REMARKS

Claims 1-5 remain pending. Claims 1, 3 and 5 are amended to advance the prosecution of this application.

The amendments to the claims are fully supported by the specification and contain no new matter. In particular, the amendments to claims 1, 3 and 5 relating to fetal tolerization are supported by the instant specification at paragraphs [0069], [0071], [0153], [0177] and [0200]. The amendment to claim 1 relating to Hepatitis C Virus (hereafter, "HCV") replication is supported by the specification at paragraphs [0101], [0102], [0200] – [0205] and Figures 31 and 32 (described in paragraphs [0055] and [0056], respectively). The amendment to claim 5, specifying transplantation after the mammal is born, is supported by the specification at paragraph [0075] and [0200].

1. The Specification Supports Mammals Having A "Normal Immune System"

Applicants respectfully direct the Examiner's attention to the following portions of the originally filed specification that support the phrase "wherein the mammal has a normal immune system."

For example, the Summary of the Invention states that "the present invention provides for a method of preparing a non-human animal having a liver comprising human hepatocytes, comprising [ ] inducing tolerance in an **immunocompetent** host non-human animal." (Specification at paragraph [0021]) (emphasis added).

In paragraph [0023], the specification teaches that "[i]mmunocompetent chimeric animals of the invention exhibit the further advantage of having **an immune system which is intact** but for exhibiting tolerance toward the human cells comprised in the animal's liver." (emphasis added).

Furthermore, when discussing the disclosed animal model systems for liver disease, the specification recognizes that it is "advantageous that the **host animal has an immune system that is intact** (but for the induced tolerance to the host cells), in that the animal's immune response to the infectious agent and/or infected human hepatocytes may produce a more accurate model of human liver diseases in which the immune system plays a pathogenic role." (Specification at paragraph [0092]) (emphasis added). Additionally, the examples demonstrate that Applicants reduced to practice an animal model in which the animal has a normal immune system and was tolerized so as to be able to support the survival of transplanted human hepatocytes.

At least by the above-recited portions of the specification, Applicants respectfully assert that the phrase "wherein the mammal has a normal immune system," recited in amended Claim 1, is fully supported by the originally filed specification, claims and drawings.

Accordingly, the "tolerized" animals of the invention do not include animals which are generally immunosuppressed, immunocompromised or immunodeficient. As such, rejections based on references relating to generally immunodeficient, immunosuppressed and/or immunocompromised animals should be removed, including the rejection under 35 U.S.C. §102(e) over United States Patent No.

6,034,297 by Vierling ("Vierling") and the rejections under 35 U.S.C. §103(a) over Rhim et al. (1995) and Vierling and over WO 96/39810 (Knudsen, 1996) in view of Vierling.

Because the rejection under the judicially created doctrine of obviousness-type double patenting has been obviated by the submission of a terminal disclaimer, the sole remaining rejection is that made under 35 U.S.C. §102(e) over United States Patent Application No. 2001/0007153 A1 by Brown et al. ("Brown").

2. Brown Does Not Anticipate (Or Render Obvious) The Claims

The Examiner maintained during the interview that Brown generally discloses an animal model for HCV infection which can be created by introducing human hepatocytes into a normal animal via, for example, intrasplenic, intrahepatic, intraportal vein or intraceliac artery injection. Applicants asserted that Brown's disclosure was insufficient to effectively tolerize a normal animal and successfully transplant and sustain xenogeneic human hepatocytes in the host liver. In response, the Examiner proposed that one of skill in the art would ascertain the necessary procedures and values by routine experimentation. Applicants respectfully disagree and address each of the Examiner's concerns below.

As pointed out during the interview by Applicants' attorney and in Applicants' previously submitted Reply, the focus of Brown is animals which are generally immunodeficient, immunosuppressed, and immunocompromised. The portion relied upon in the pending rejection provides only the most general reference to the use of

animals that are tolerized to "a specific antigen or set of antigens while preserving a capability of immune response to other antigens." In particular, Brown states (at paragraph [0044]):

> A number of systems can be used in the present invention to allow tolerance to a specific antigen or set of antigens while preserving a capability of immune response to other antigens. These can include but are not limited to neonatal tolerance, thymic tolerance, T cell depletion or inactivation and oral tolerization as well as combinations thereof. Means of carrying out these methods are described in U.S. patent application Ser. No. 08/806,629, supra.

Note first that the above disclosure completely omits reference to **fetal tolerization**. As will be discussed further below, fetal tolerization provides a particular advantage in the preparation of animals of the invention. The claims are presently amended to require that mammals be rendered tolerant as fetuses.

Second, Applicants invite the Examiner's attention to the patent application referred to in the cited paragraph 44 of Brown. Searches by Attorneys for Applicants have indicated that the cited application is abandoned. Applicants have now, however, identified United States Patent Application Publication No. US 2004/0023909A1 ("the '909 application") by Roy-Chowdhury et al., which is a continuation of an application which is a divisional of U.S.S.N. 08/808,629, the cited application. The relationship between U.S.S.N. 08/808,629 and the '909 application suggests that their disclosures are the same.

The disclosure of the '909 application is not, as might appear from Brown paragraph [044], a reference for means of eliciting neonatal tolerance, thymic tolerance, T cell depletion or inactivation and oral tolerization. Rather, it relates to "selective

immune down regulation" ("SIDR"), and specifically addresses fetal tolerization only in its background section, where it is contrasted to the purported invention. Specifically, the '909 application, at paragraph [0007], states:

> In contrast to general immunosuppression, tolerance to specific antigens (such as adenovirus particles) can be produced if the antigen is injected into a neonate or into a fetus [citations]. However, this procedure has a major limitation. It is effective only in the fetus or during the first few days after birth (not an adult).

Attorneys for Applicants enclose herewith, as Exhibit A, a copy of the '909 application.

Thus, Brown does not disclose fetal tolerization, and the application that it cites appears, from disclosure available to Applicants, to teach away from fetal tolerization.

The superficial mention by Brown, that animals may be tolerized to accept hepatocyte transplants, does not enable a chimeric non-human animal of the present invention, as claimed, which is *sufficiently* tolerized to the presence of human hepatocytes to qualify as a valid HCV model. In this regard, it is important that the immune response of the animal to human hepatocytes not merely be curtailed, but that it be curtailed sufficiently to allow the human hepatocytes to persist and be assimilated into the host animal sufficiently to permit HCV infection to be sustained. This important feature of the invention, that HCV replication occur, has been added to the claims by amendment.

Applicants therefore assert that the claims, as amended, are not anticipated by Brown, such that the pending rejection should be withdrawn.

In addition, Applicants assert that the claims are not rendered obvious by Brown. In the laboratory of the present inventors, it has been found that fetal tolerization offers a substantial advantage over other tolerization methods. As evidence of this advantage, Applicants invite the Examiner's attention to Wu et al., 2001, Croatian Medical Journal 42(4):446-450, enclose herewith as Exhibit B ("Wu 2001").

Figure 2 of Wu 2001, at page 447, shows a comparison of the amount of tolerization achieved (as measured by mixed lymphocyte assay ("MLA")), relative to control levels, by fetal (bar 2), intra-thymic (neonatal) (bar 3) and oral (neonatal) (bar 4) tolerization, relative to the response of a non-tolerized rat stimulated with irradiated human hepatocytes (bar 1). It can be seen that the MLA response of cells from the fetally tolerized animal is close to control (baseline), 100%. The responsiveness of animals tolerized as neonates was significantly greater. Thus, animals supposedly "tolerized" postnatally continued to produce an immune response against human hepatocytes, a real disadvantage when a stable model system that sustains HCV infection is the goal.

As evidence of the unexpected success of fetal tolerization in producing a stable HCV model system, Applicants invite the Examiner's attention to Exhibit C, a manuscript entitled "A NOVEL IMMUNOCOMPETENT RAT MODEL OF HCV INFECTION AND HEPATITIS" by the inventors ("Wu 2004"), which will be submitted for publication in the near future. As stated in the present application at paragraphs [0010] – [0016], HCV produces a devastating disease in humans, and no satisfactory model system has hitherto been developed. Wu 2004 shows that, using the methods of

the invention, a HCV model system was produced with the following characteristics (see Abstract):

> Fluorescent antibodies against human albumin in frozen liver sections demonstrated the presence of Huh 7 cells in the liver. HCV viral replication was demonstrated in livers of tolerized, transplanted and HCV infected rats by the presence of HCV RNA bands of the expected size from nested PCR using negative strand specific primers. Levels of HCV in serum were measured by real time PCR at 7,500 copies/ml at week 5, rose to 25,000 copies/ml by week 13, and remained at that level through the 16th week. In tolerized, transplanted, inoculated rats, but not controls, serum alanine aminotransferase (ALT) values rose from normal prior to inoculation, to a peak at 120 IU/L at 13 weeks and remained elevated through the duration of the experiment. Histology showed foci of mononuclear infiltrates in portal and central regions only in tolerized, transplanted rats, but not controls. *Conclusions:* HCV-inoculated immunocompetent rats tolerized and transplanted with human Huh 7 cells support HCV gene expression, RNA replication, and developed biochemical as well as histological evidence of hepatitis.

As the Examiner can appreciate, the study of HCV has been hampered by the lack of appropriate model systems. In particular, it is important that such model systems have intact immune systems because the host immune system plays a leading role in the pathogenesis of HCV. Neither Brown nor any other reference of record has taught or demonstrated an animal model system that faithfully mimics HCV infection of human hepatocytes. By contrast, the presently claimed model system has demonstrated HCV infection of human hepatocytes in an animal with a normal immune system.

Accordingly, Applicants assert that the perfunctory disclosure of Brown regarding tolerization, alone or in combination with other references, neither would anticipate nor render obvious the HCV model system of the invention.

Conclusion

Applicants thank the Examiner for her helpful comments on the present application. Should another discussion assist in advancing prosecution of this application, the Examiner is invited to call the undersigned.

Respectfully submitted,

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Enclosures